HIV Vaccines

"Neutralization Profile of Sera from HIV Infected Patients to Autologous HIV Isolates and MN Strain."

1996 FEB 26 -- According to an abstract submitted by the authors to the 3rd Conference on Retroviruses and Opportunistic Infections, held January 28 - February 1, 1996, in Washington, DC, "Sera from HIV infected patients (antiretroviral therapy naive) were examined for their neutralizing activity (NA) against autologous isolates and to HIV strain MN using a PBMC-based infectivity reduction assay. The absolute $CD4^+$ cell count ranged between 116-422 cells/(mu)L. Sera from 4 patients significantly neutralized both autologous and MN strain (+/+) by 99.2-99.9% and 81-98% respectively. Three sera neutralized only MN but not the autologous isolate (-/+) by 96-98.9% and 3 sera failed to neutralize either strain (-/-, double -ve). There was no correlation between the NA profile and the CD4 count, the SI/NSI phenotype of the HIV isolates or the tropism to lymphocytes and macrophages. However the occurrence of the three double -ve neutralizations correlated with the virus load (IUPM) of the PBMC from those patients as determined by HIV quantitative microculture assay. The IUPM from those patient were 2043 ± 798 compared to 241 ± 114 for the rest of the group. The neutralization profile to autologous and MN strains might be useful for selecting candidate patients for immunoglobulin or vaccine therapy."

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HIV-1 gp120 Stimulates the Production of β-Chemokines in Human Peripheral Blood Monocytes Through a CD4-Independent Mechanism[1]

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The present study was designed to evaluate the effect of the HIV-1 envelope glycoprotein gp120 on the expression of β-chemokines in cultured monocytes/macrophages. Treatment of either freshly isolated 1-day-cultured monocytes or 7-day-cultured monocyte-derived macrophages (MDM) with recombinant gp120-IIIB resulted in a specific and dose-dependent enhancement of secretion of monocyte chemoattractant protein-1, macrophage inflammatory protein-1β, and RANTES as well as a clear-cut increase in transcript accumulation. The expression of these mRNA was increased, but not superinduced, in the presence of cycloheximide. β-Chemokine secretion was also induced after exposure of monocyte cultures to gp120-JRFL and aldrithiol-2-inactivated R5 and X4 HIV-1 strains, retaining conformational and functional integrity of envelope proteins. In contrast, no β-chemokine secretion was triggered by X4 and R5 gp120 or aldrithiol-2-inactivated virus treatment of monocytoid cell lines that were fully responsive to LPS. The gp120-mediated effect was independent of its interaction with CD4, as preincubation with soluble CD4 did not abrogate β-chemokine induction. Moreover, triggering of CD4 receptor by a specific Ab did not result in any β-chemokine secretion. Interestingly, engagement of CCR5 and CXCR4 receptors by specific Abs as well as treatment with CCR5 and CXCR4 ligands induced β-chemokine secretion. On the whole, these results indicate that HIV-1 stimulates monocytes/macrophages to produce β-chemokines by a specific interaction of gp120 with HIV-1 coreceptors on the cell membrane. The expression of these related polypeptides may represent an important cellular response for regulating both the extent of viral infection and the recruitment of immune cells. *The Journal of Immunology*, 2001, 166: 5381–5387.

Human peripheral blood monocytes mature into different types of tissue histiocytes when they migrate from the bloodstream to various tissues. Directional migration of monocytes is regulated by locally produced cell-secreted proteins, bacterial peptides, and products of phospholipid metabolism (1–3). In the past few years a novel superfamily of low m.w. chemotactic proteins has been described (2, 3). These related polypeptides are further grouped into two main subfamilies, the C-X-C (α-chemokines) and the C-C (β-chemokines) families, according to their arrangement of cysteine residues. The α-chemokines are primarily active on neutrophils, but some activity has also been reported on T lymphocytes (4). In contrast, the β-chemokines exhibit a wide spectrum of action, in that they are active on multiple leukocyte populations, including monocytes, granulocytes, T lymphocytes, NK, and dendritic cells (5, 6). Recent evidence has clearly shown that chemokines and their receptors may play an important role in the regulation of HIV-1 infection (7, 8). In particular, α (i.e., stromal derived factor-1 (SDF-1)[3]) and β-chemokines (i.e., RANTES, macrophage inflammatory protein (MIP)-1α, MIP-1β, and monocyte chemoattractant protein (MCP)-3) have a potent suppressive

effect on HIV-1 infection resulting from an early postbinding block in virus fusion and entry (9–17). Interestingly, some of these factors have also been reported to have a stimulating effect on HIV attachment, replication, and cell-mediated transmission (18–22). However, the in vivo role of β-chemokines in the pathogenesis of HIV disease remains to be fully elucidated. Some studies have also shown that changes in the production of specific chemokines occur in the course of HIV infection. In this regard, an increased production of MIP-1α, MIP-1β, RANTES, and MCP-1 has been observed in macrophages infected in vitro with HIV-1 (23–26). Likewise, elevated levels of some chemokines have been found in the CSF of patients with HIV encephalitis, suggesting the involvement of these molecules in HIV-related and associated pathology of the nervous system (27–30). The stimulation of chemokines production by HIV infection has been observed in the presence (23, 24, 26) and in the absence (25) of active viral replication. Although it has been reported that the addition of exogenous Tat protein induced MCP-1 production in astrocytes (29) as well as in peripheral blood monocytes (26), little information is currently available on the roles of other viral products.

In this study we report that the treatment of monocytes/macrophages with HIV-1 gp120 as well as with inactivated R5 and X4 HIV-1 strains results in the production of MCP-1, MIP-1β, and RANTES. Notably, β-chemokine secretion is also induced following engagement of CCR5 and CXCR4, but not CD4 receptors, by specific Abs or ligands. These results indicate that early interactions between monocyte/macrophage cell surface and HIV external components can stimulate β-chemokine secretion in the absence of a productive infection. The expression of these different, but related, polypeptides after stimulation of monocytes/macrophages with HIV-1 may represent an important response for regulating both viral spreading and the type of immune cells recruited during the course of viral infection.

Materials and Methods

Isolation and culture of peripheral blood monocytes

PBMC were obtained from 18- to 40-year-old healthy men as previously described (31). Monocytes were separated from lymphocytes by Percoll gradient centrifugation (32). Cells were then cultured in endotoxin-free Iscove's medium containing 15% FCS (0.22-μm pore size filter) for 24 h (defined here as 1-day monocytes) or 7 days (7-day monocyte-derived macrophages (MDM)). Cytochemical (i.e., sodium fluoride-inhibited esterase activity) and surface marker (i.e., CD14 Ag) analysis revealed that the adherent cell population consisted of >95% monocytes.

Cell lines

U937 cells, a well-known human promonocytic cell line, were cultured in RPMI 1640 containing 10% heat-inactivated FCS. THP-1 cells were cultured in the same medium containing 5×10^{-5} M 2-ME.

Reagents

LPS and cycloheximide (CHX) were purchased from Sigma (St. Louis, MO). mAb to human CD4 (SIM 4, catalog no. 724) and soluble CD4 (catalog no. 1813) were provided by the National Institutes of Health AIDS Research and Reference Reagent Program. Recombinant HIV-1 gp120 (strain IIIB) was purchased from Intracel (London, U.K.) and ImmunoDiagnostics (Bedford, MA). Recombinant HIV-1 gp120 (strain JRFL) was provided by R. Doms. mAbs to human CCR5 (clone 45529.111) and CXCR4 (clone 44717.111) were purchased from R&D Systems (Minneapolis, MN). SDF-1α, SDF-1β, and MIP-1α were purchased from PeproTech (London, U.K.). LPS contamination of reagents was excluded by checking their endotoxin activity by the Limulus amebocyte assay (Charles River Endosafe, Charleston, SC; detection limit, 0.125 endotoxin U/ml). The endotoxin content determined in gp120-IIIB, gp120-JRFL, and Ab to CXCR4 and CCR5 preparations was <0.125 endotoxin U/ml. The gp120 was inactivated by boiling the protein for 30 min.

Virus inactivation procedure

For HIV-1 IIIB and BaL inactivation, a 100 mM stock solution of the compound 2,2′-dithiodipyridine (aldrithiol-2; AT-2) was prepared and added directly to viral stocks at a final concentration of 1 mM. Virus preparations were treated for 1 h at 37°C and then kept on ice for 2 h. At the end of the inactivating procedure, treatment agent was removed by ultracentrifugation at 17,000 × g for 1 h at 4°C. Viral pellets were resuspended in endotoxin-free Iscove's medium containing 15% FCS.

Measurements of MCP-1, MIP-1β, and RANTES

The levels of MCP-1, MIP-1β, and RANTES present in culture supernatants were measured using ELISAs. ELISA kits were purchased from R&D Systems. The detection limit was 5 pg/ml for MCP-1 and RANTES and 4 pg/ml for MIP-1β.

RT-PCR detection of MCP-1, MIP-1β, and RANTES mRNA

Total RNA was extracted by the method of Chirgwin (33) and reverse transcribed as previously described (31). A 1/10 dilution of the cDNA product was amplified in a 20-μl reaction mixture (31) containing 0.5 U of Taq polymerase (Perkin-Elmer, Foster City, CA). Amplification (40 s at 94°C, 1 min at 62°C, and 1 min at 72°C) was performed for 17–20 cycles with MCP-1 primers and for 20–25 cycles with GAPDH, RANTES, and MIP-1β primers; amplification was preceded by a denaturation step (3 min at 94°C) and was followed by a final extension period (10 min at 72°C). PCR products were analyzed by 2.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The sequences of the GAPDH, MCP-1, MIP-1β, and RANTES primers have been previously described (24, 31, 34, 35).

Statistical analysis

Statistical analysis of data was performed using the nonparametric Kruskal-Wallis test. Values of $p < 0.05$ were considered significant.

Results

Induction of MCP-1, MIP-1β, and RANTES by HIV-1 gp120

We have investigated the effect of X4 gp120 (HIV-1 IIIB strain) on the production of β-chemokines in monocytes at different stages of differentiation. As shown in Fig. 1, 1-day monocytes spontaneously released variable amounts of MIP-1β and, to a greater ex-
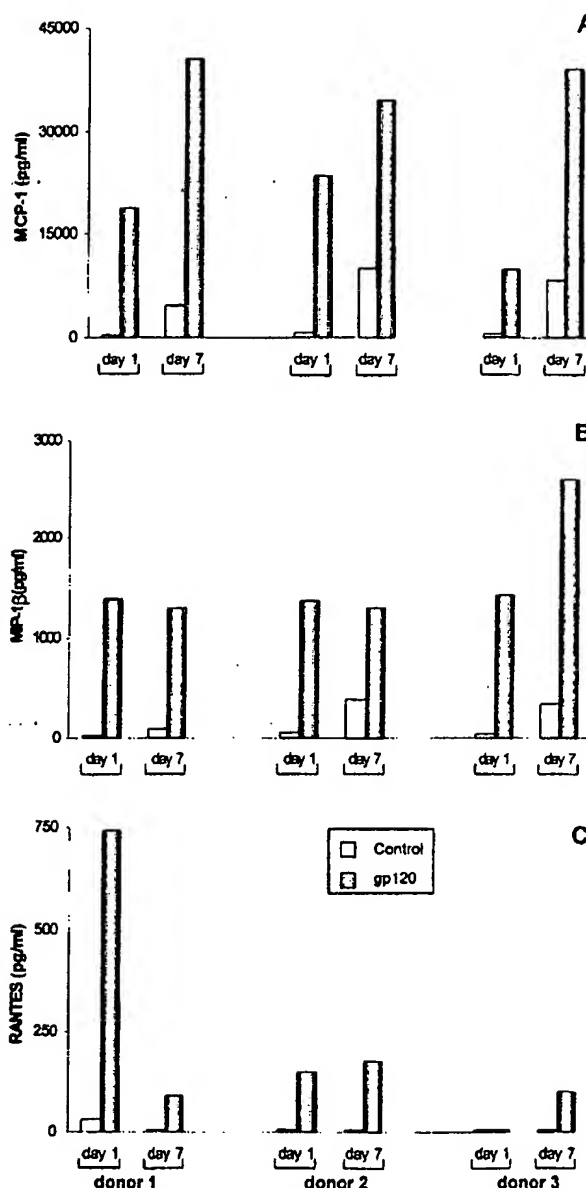

FIGURE 1. Secretion of β-chemokines by monocytes/macrophages treated with HIV-1 gp120 protein. Monocytes isolated from the peripheral blood of healthy donors were seeded in 48-well cluster plates at a density of 6.25×10^5 cells/cm². After 1 or 7 days of culture, cells were treated with gp120-IIIB (1 μg/ml) or were left untreated. After 24 h of culture, supernatants were harvested and frozen before MCP-1 (A), MIP-1β (B), or RANTES (C) determination. Results obtained with three different donors are shown. Comparable results were obtained using recombinant gp120 from different sources (Intracel and ImmunoDiagnostics; data not shown). Data are the mean values of duplicate samples. Statistical analysis showed a significant increase ($p < 0.05$) in MCP-1, MIP-1β, and RANTES levels in gp120-treated cultures compared with controls. The intersample SD did not exceed 10%.

tent, MCP-1. The secretion of these chemokines was generally increased during in vitro differentiation. In contrast, barely detectable levels of RANTES were occasionally found in monocytes/macrophages independently of the differentiation stage. The addition of HIV-1 gp120 to monocytes/macrophages markedly enhanced the secretion of MCP-1 and MIP-1β and, to a lesser

extent, RANTES compared with unstimulated cultures (Fig. 1). The capacity of monocytes/macrophages to secrete β-chemokines in response to gp120 was not related to their differentiation state, as comparable levels of induction were found in 1-day monocytes and 7-day MDM. Similar results were obtained with R5 gp120 (JRFL strain; data not shown). Experiments were then conducted to evaluate the minimal amount of gp120 required to induce β-chemokine secretion in 7-day MDM. As shown in Fig. 2, gp120 induced MCP-1, MIP-1β, and RANTES secretion in a dose-dependent manner. Macrophages from all donors consistently secreted MCP-1 and MIP-1β in response to gp120 concentrations of 1000

and 300 ng, respectively, whereas only the higher gp120 concentration (1000 ng/ml) induced RANTES secretion in all donors. At lower doses (100 and 30 ng/ml) no significant induction was observed (Fig. 2).

Specificity of gp120-induced secretion of β-chemokines

As LPS is an inducer of β-chemokines (36), we first excluded any detectable presence of this molecule in our gp120 preparations using the *Limulus* amebocyte assay. Notably, gp120 was even more effective than LPS in inducing the secretion of MCP-1 and MIP-1β (Table I). Moreover, no induction of chemokine secretion was detected after treatment with heat-inactivated gp120. In this regard, it is worthwhile to mention that LPS is thermostable and not inactivated at 120°C for 30 min (37). The capacity of gp120 to induce β-chemokine secretion was not restricted to X4 gp120 (IIIB strain), as similar results were obtained after treatment with R5 gp120 (JRFL strain; Table I). To further support the specificity of gp120-induced β-chemokine production, 7-day MDM were exposed to AT-2-inactivated HIV-1 virions. In contrast to conventional methods of inactivation (i.e., heat or formalin treatment), this inactivation procedure allows viruses to retain conformational and functional integrity of viral surface proteins (38). As shown in Table I, exposure of 7-day MDM to AT-2-inactivated R5 and X4 HIV-1 strains resulted in a marked induction of MCP-1 and MIP-1β secretion and, to a lesser extent, RANTES.

Effect of gp120 on the production of β-chemokines in monocytic cell lines

Previous studies have shown that β-chemokine production can be induced in monocytic cell lines in response to HIV infection (26). Therefore, experiments were conducted to establish whether gp120 treatment could induce β-chemokine secretion in some monocytic cell lines. As shown in Table II, although LPS induced a clear-cut secretion of MCP-1, MIP-1β, and RANTES in THP-1 and U937 cells, no increase over the basal levels of these chemokines was detected after gp120 treatment, indicating a specificity of the gp120 effect on primary monocytes.

Regulation of MCP-1, MIP-1β, and RANTES transcripts expression by gp120

Experiments were then conducted to investigate whether the increased secretion of β-chemokines induced by gp120 was associated with an enhanced accumulation of the corresponding mRNA. As shown in Fig. 3, gp120 induced a clear-cut accumulation of MCP-1, MIP-1β, and RANTES mRNA in 7-day MDM. In addition, a typical protein synthesis inhibitor, such as CHX, also induced a marked increase in the steady state levels of MCP-1, MIP-1β, and RANTES transcripts. However, the simultaneous addition of CHX and gp120 did not further induce mRNA accumulation.

Roles of CD4 and chemokine receptors in the gp120-mediated induction of β-chemokines

To establish the relative importance of gp120 interactions with CD4 and chemokine receptors to the gp120-induced secretion of β-chemokines, the effects of Abs directed against CD4, CXCR4, and CCR5 receptors were investigated. As shown in Fig. 4, the CD4 engagement by a specific Ab mimicking gp120 binding did not result in any MCP-1, MIP-1β, and RANTES induction in 7-day MDM. In addition, preincubation of gp120-IIIB with soluble CD4 did not abolish the ability of gp120 to induce β-chemokine secretion. Similar results were obtained when gp120-JRFL was used (data not shown). On the contrary, the engagement of the chemokine receptors CXCR4 and CCR5 by means of specific Abs resulted in a clear-cut induction of MCP-1, MIP-1β, and, to a
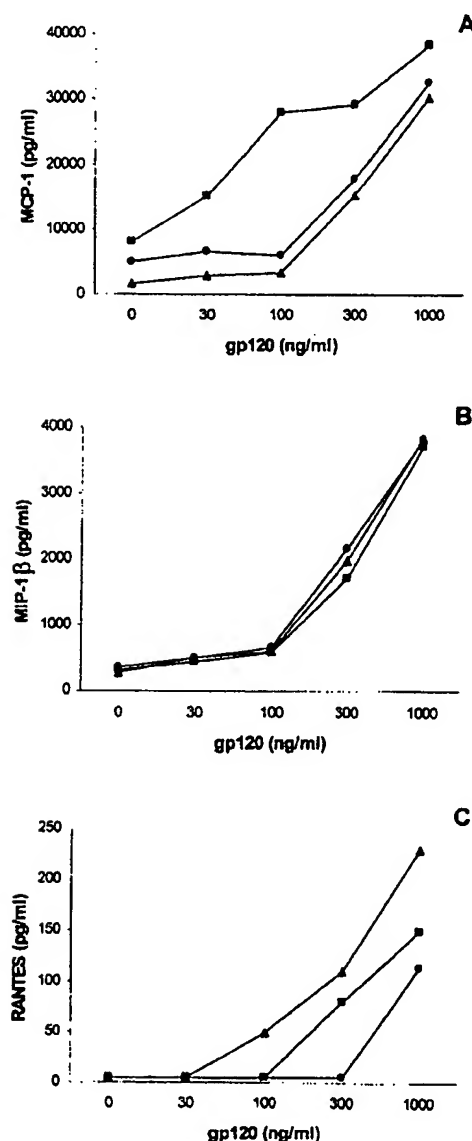
FIGURE 2. Dose response of gp120-induced β-chemokine secretion in monocytes/macrophages. Monocytes were seeded as described in Fig. 1. After 7 days of culture, cells were treated with different concentrations of gp120-IIIB (ranging from 30 to 1000 ng/ml) or were left untreated. Twenty-four hours later, supernatants were collected for MCP-1 (*A*), MIP-1β (*B*), or RANTES (*C*) determination. Results obtained with three different donors are shown (●, donor 1; ■, donor 2; ▲, donor 3). Data are the mean values of duplicate samples. The intersample SD did not exceed 10%.

Table I. *Specificity of gp120-induced secretion of β-chemokines in macrophages[a]*

Treatment	MCP-1 Production (pg/ml)					MIP-1β Production (pg/ml)					RANTES Production (pg/ml)				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
None	8,085	9,120	11,300	9,800	7,200	348	980	264	375	200	<31.2	<31.2	<31.2	<31.2	<31.2
LPS	26,550	ND	34,360	33,800	25,400	2,300	ND	1,300	2,150	1,115	370	ND	340	1,010	530
gp120-IIIB	38,910	44,600	57,800	41,570	17,000	3,900	4,000	3,899	2,900	2,300	270	500	154	117	70
Heat-inactivated gp120-IIIB	11,750	13,750	ND	ND	ND	800	1,100	ND	ND	ND	<31.2	<31.2	ND	ND	ND
gp120-JRFL	ND	ND	ND	30,800	36,800	ND	ND	ND	2,625	2,850	ND	ND	ND	375	115
AT-2-inactivated HIV-1 IIIB	ND	29,240	44,850	ND	ND	ND	3,771	4,053	ND	ND	ND	40	<31.2	ND	ND
AT-2-inactivated HIV-1 BaL	ND	51,120	16,535	ND	ND	ND	3,976	814	ND	ND	ND	101	35	ND	ND

[a] Monocytes were cultured as described in Fig. 1. After 7 days, cells were treated with LPS (100 ng/ml), gp120-IIIB (1 μg/ml), heat-inactivated gp120-IIIB (1 μg/ml), gp120-JRFL (1 μg/ml), AT-2-inactivated HIV1 IIIB (multiplicity of infection = 0.1), or AT-2-inactivated HIV-1 BaL (multiplicity of infection = 0.1). After 24 h of culture, supernatants were harvested and tested for MCP-1, MIP-1β, and RANTES content. Each value represents the mean of duplicate culture samples. Intersample SD did not exceed 10%.

lesser extent, RANTES secretion in 7-day MDM, suggesting a role for gp120-coreceptor interactions in the induction of β-chemokine secretion. To provide further evidence for the involvement of chemokine receptors in the gp120-mediated β-chemokine secretion, the effects of CXCR4 and CCR5 ligands were analyzed. As shown in Fig. 4, treatment of 7-day MDM with SDF-1α and SDF-1β, specific CXCR4 ligands, resulted in the induction of MCP-1, MIP-1β, and RANTES secretion. Moreover, treatment of 7-day MDM with MIP-1α, a chemokine interacting with CCR5, resulted in the induction of MCP-1 and MIP-1β, but not RANTES, secretion. This chemokine was chosen among others capable of binding to CCR5, including RANTES and MIP-1β, as its addition to monocyte cultures would not have interfered with subsequent RANTES and MIP-1β determinations. Furthermore, a similar induction of MCP-1 was obtained by treating macrophages with RANTES or MIP-1β, which also bind to CCR5 (data not shown).

Discussion

Macrophages are major targets for HIV infection and play an important role in the pathogenesis of AIDS (39). These cells represent major sources for the production of β-chemokines, including MCP-1 and MIP-1β, which are secreted in response to inflammatory stimuli and, at the same time, are important targets of chemokine action (1-6). Chemokines are key factors affecting the directional migration of immune cells. Because altered patterns of cellular trafficking may influence the spread of infection, it can be envisaged that HIV-induced changes in the physiological levels of

chemotactic factors play an important role in the pathogenesis of AIDS.

In this study we have reported for the first time that HIV-1 gp120 protein, derived from either X4 (IIIB) or R5 (JRFL) HIV-1 strains, is a potent inducer of β-chemokine secretion, including MCP-1, MIP-1β, and RANTES, in primary human monocytes/ macrophages through a CD4-independent mechanism. Exposure of monocyte cultures to AT-2-inactivated R5 and X4 HIV-1 strains also results in a consistent secretion of β-chemokines. Notably, this method of inactivation allows the envelope proteins to retain their conformational and functional integrity, and viral infectivity is completely abrogated by subsequent modification of the nucleocapsid protein (38). A number of studies have previously shown an enhanced production of MIP-1α, MIP-1β, and MCP-1 in HIV-1-infected macrophage cultures concomitantly with maximal virus production (23, 24, 26). HIV replication was requested for this effect, as demonstrated by the absence of chemokine up-regulation after infection in the presence of 3'-azido-3'-deoxythymidine (23, 24, 26). In contrast, some studies reported the induction of chemokine secretion in the absence of active viral replication as well as in uninfected cells exposed to HIV products. In particular, viral replication was not required for the up-regulation of RANTES production observed in HIV-infected PBMC (25). Likewise, induction of β-chemokine secretion was observed in macrophages (26) and astrocytes (29) stimulated with exogenous Tat. We have previously reported increased expression of an α-chemokine (i.e., IL-8) in monocytes/macrophages treated with gp120 (40). Moreover, it

Table II. *Effect of LPS, gp120, and AT-2-inactivated HIV-1 on the production of β-chemokines in monocytic cell lines[a]*

Treatment	MCP-1 Production (pg/ml)		MIP-1β Production (pg/ml)		RANTES Production (pg/ml)	
	U937	THP-1	U937	THP-1	U937	THP-1
None	1,570	<31.2	<15.6	<15.6	350	400
LPS	18,700	260	280	2,850	755	2,140
gp120IIIB	1,630	<31.2	<15.6	<15.6	390	300
gp120JRFL	1,495	<31.2	<15.6	<15.6	415	320
Inactivated HIV-1 IIIB	1,515	<31.2	<15.6	<15.6	250	290
Inactivated HIV-1 BaL	1,730	<31.2	<15.6	<15.6	300	350

[a] U937 and THP-1 were plated in 48 well-cluster plates at the concentration of 4 × 10⁵/ml in RPMI 1640 containing 10% FCS. Cells were treated for 24 h with LPS (100 ng/ml), gp120-IIIB (1 μg/ml), gp120-JRFL (1 μg/ml), AT-2-inactivated HIV1 IIIB (multiplicity of infection = 0.1), or AT-2-inactivated HIV-1 BaL (multiplicity of infection = 0.1). Then, the supernatants were collected for MCP-1, MIP-1β, and RANTES determination. Each value represents the mean of duplicate culture samples. Intersample SD did not exceed 10%.
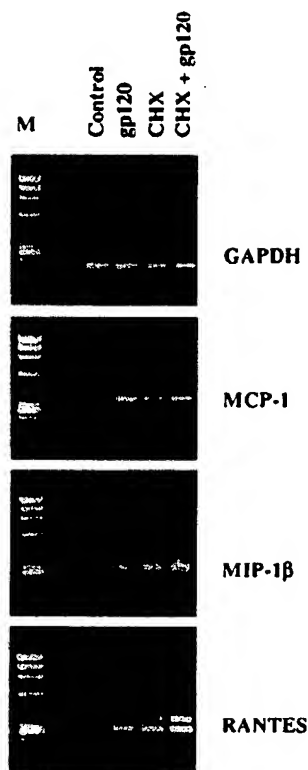
FIGURE 3. Effects of gp120 and CHX on the expression of MCP-1, MIP-1β, and RANTES mRNA in monocytes/macrophages. Monocytes were seeded in six-well cluster plates at a cell density of 2×10^5 cells/cm^2. After 7 days of culture, cells were treated with gp120-IIIB (1 μg/ml) or CHX (10 μg/ml) for 4 h. Total RNA was extracted as described in *Materials and Methods*. MCP-1, MIP-1β, and RANTES mRNA expressions were analyzed by RT-PCR assay as described in *Materials and Methods*. PCR experiments were run for 17 cycles for MCP-1 and for 20 cycles for MIP-1β and RANTES. This low number of amplification cycles ensures the detection of mRNA bands under conditions of linear amplification as assessed in exploratory experiments. However, a basal level of mRNA expression can be detected for all β-chemokines by increasing the number of cycles up to 20–25 depending on the donor. A representative experiment of three is shown.

FIGURE 4. Roles of CD4 and chemokine receptors in the gp120-mediated induction of β-chemokines. Monocytes were seeded as described in Fig. 1. After 7 days of culture, cells were treated with gp120-IIIB (1 μg/ml); not membrane bound CD4 (2 μg/ml); gp120-IIIB plus sCD4 (preincubated at 37°C for 30 min); Abs to CD4 (1 μg/ml), CCR5 (5 μg/ml), or CXCR4 (5 μg/ml); MIP-1α (300 ng/ml); and SDF-1α (200 ng/ml) plus SDF-1β (200 ng/ml). After 24 h of culture, supernatants were harvested and frozen before MCP-1 (A), MIP-1β (B), or RANTES (C) determination. Data are the mean values of duplicate samples. The intersample SD did not exceed 10%. A representative experiment of three is shown.

has been shown that HIV-2 external glycoproteins that are involved in the early virus-host interaction can induce the secretion of some β-chemokines (i.e., MIP-1α, MIP-1β, and RANTES) in total PBMC, although the cell types involved in this effect have not been characterized (41). The ensemble of these results, apparently contradictory, suggests that chemokine induction during the course of HIV infection can be triggered by different mechanisms. In productively infected cells, the active viral replication can play a role in triggering chemokine secretion, which generally occurs concomitantly with the peak of viral production. In contrast, bystander cells can be stimulated to secrete chemokines by soluble viral products, such as Tat and gp120, locally released from the infected cells. In this regard, it is worth mentioning that gp120 is released into the circulation of HIV-infected subjects, and it is thought to have a role in the progressive immune derangement observed in these patients (42). The availability of gp120 in AIDS patients has been documented in a number of reports, either as free protein or complexed by anti-gp120 Ab, suggesting that there could be ample opportunity for virus-associated or shed gp120 to interact with surface components of immune cells. In particular,
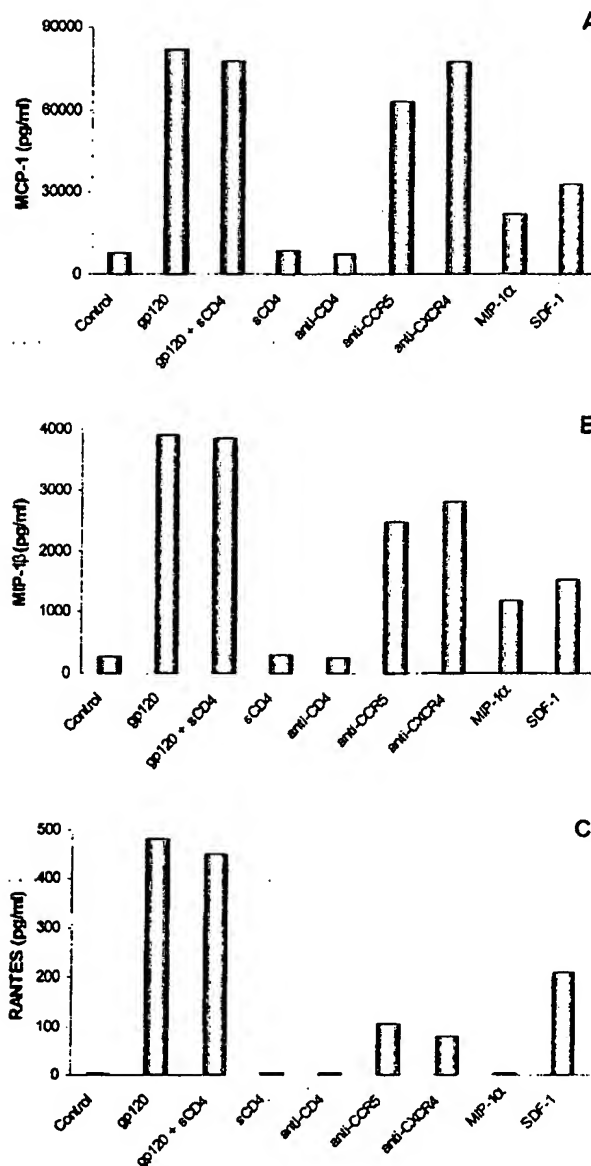
Oh and coworkers (43) found that the amount of gp120 released in the serum of HIV-infected patients ranged from 12 to 92 ng/ml. Notably, we report that as little as 300 ng/ml of gp120 significantly enhanced the production of MCP-1 and MIP-1β in all donors assessed, whereas slightly higher concentrations of gp120 were needed for consistent secretion of RANTES. We envisage that gp120 concentrations suitable for the induction of at least some chemokines can be locally achieved in vivo, thus contributing to the up-regulation of β-chemokine production observed in the

course of HIV infection. Interestingly, the capacity of gp120 to induce β-chemokine secretion appears to be restricted to primary monocytes/macrophages. In fact, our results clearly indicate that although monocytic cell lines are fully responsive to LPS in terms of β-chemokine secretion, the production of β-chemokines induced by R5 and X4 recombinant gp120 occurs only in primary macrophages. Likewise, no β-chemokine secretion was observed after exposure to AT-2-inactivated R5 and X4 HIV-1 strains of monocytic cell lines (Table II). These results represent a further example of the remarkable differences frequently observed between primary monocytes/macrophages and established monocytic cell lines in terms of functional responses to HIV infection.

The results of the RT-PCR experiments described in this article indicate that gp120 induces MCP-1, MIP-1β, and RANTES mRNA accumulation over the basal levels normally expressed in macrophages. Although a clear-cut increase in these transcripts was observed in the presence of CHX, treatment of macrophages with gp120 in the presence of this protein synthesis inhibitor did not result in any further accumulation of β-chemokine mRNA. These results suggest that MCP-1, MIP-1β, and RANTES transcripts, although constitutively transcribed, can undergo a rapid turnover. Moreover, the response of these cells to gp120 does not require de novo protein synthesis. This primary response may be due to an enhancement of the basal level of transcription or to a stabilization of MCP-1, MIP-1β, and RANTES mRNA, both of which would result in the accumulation of these transcripts. Additional experiments are needed to precisely define the mechanism of action of gp120 in the regulation of β-chemokine mRNA expression.

Much attention has been focused on how the gp120 envelope protein may interact with chemokine receptors, and considerable information has been generated concerning the interaction of HIV-1 with CD4 and chemokine receptors on T cells and transfected cells. In contrast, the nature of the interaction of gp120 with CD4 and cofactors on monocytes/macrophages has not been carefully characterized. It has been suggested that after binding of gp120 to CD4, a conformational change endows an epitope of gp120 with the capacity to bind chemokine receptors (8). Recently, it has been demonstrated that gp120 acts as a functional ligand of CXCR4 and CCR5 in primary monocytes (44). Interestingly, distinct responses were evoked by gp120 and chemokines despite the use of the same receptors (44). Our results clearly show that the capacity of gp120 to induce β-chemokine expression is independent of its interaction with CD4 receptors. In fact, we show that preincubation of gp120 with an excess amount of soluble CD4 does not interfere with its capacity to stimulate β-chemokine secretion. In this regard, some studies have reported association of gp120 with CXCR4 in a CD4-independent manner. In particular, it has been shown that gp120 can induce apoptosis through a direct interaction with CXCR4 in neuronal (45) and CD8 T cells (46), both lacking CD4 receptor. Likewise, Liu and colleagues (44) showed that gp120-specific ionic channel activation is not caused by signaling through CD4, as no effect was induced by R5 gp120 binding in CCR5-negative MDM or in ADM3100-treated macrophages stimulated with X4 gp120. In keeping with these observations, we have also shown that a mAb (SIM4) that binds to the same epitope as Leu 3a and blocks HIV/CD4 binding and infection has no effect on β-chemokine expression. In contrast, specific Abs recognizing the CCR5 or CXCR4 receptors induce a marked secretion of MCP-1 and MIP-1β and, to a lesser extent, RANTES. Likewise, SDF-1, the natural CXCR4 ligand, and MIP-1α, one of the chemokines interacting with CCR5, also induce some secretion of β-chemokines. In addition, treatment of monocyte cultures with MIP-1β and RANTES results in a consistent secretion of MCP-1

(data not shown). Consistent with these results, both AT-2-inactivated R5 and X4 HIV-1 strains, exposing a fully functional gp120 at their surface, induce marked secretion of β-chemokines independently of their coreceptor usage.

Contrasting results have been obtained by different groups, including ours, on the expression of CXCR4 and CCR5 during the course of monocyte differentiation to macrophages. In particular, we have shown that the differentiation process is accompanied by a consistent reduction in the expression of CXCR4 and CCR5 (32). However, comparable levels of β-chemokine induction were detected in 1- and 7-day-cultured monocytes (Fig. 1). These results suggest that even low receptor expression is sufficient to induce a clear-cut response to gp120. In this regard it has been reported that differentiated macrophages expressing low levels of CXCR4 can support X4 HIV-1 strain replication (47). Likewise, it has been shown that while the majority of CCR5-using viruses do not infect T cell lines, a small subset of primary R5 HIV-1 strains is able to infect CD4$^+$ T cell lines by virtue of its capacity to exploit low levels of CCR5 for infection (48). Moreover, the involvement of CCR5 as a coreceptor for HIV-1-BaL entry has been demonstrated by chemokine competition experiments, even though CCR5 expression was undetectable by FACS analysis (49). In this regard it is of interest that different subclones of U937 cells, expressing comparable levels of functional CXCR4, efficiently support fusion with cells expressing HIV-IIIB Env, but differ in their susceptibility to the infection with X4 HIV-1 strains (50). Overall, these results strongly suggest that the levels of coreceptor expression are not the sole determinants of the response to HIV infection.

Together, our results indicate that the capacity of gp120 to induce β-chemokines in monocytes/macrophages is not restricted to specific viral strains, suggesting that this gp120-mediated effect can be operative during all stages of disease. Thus, the interaction of both X4 and R5 HIV-1 envelope glycoproteins with chemokine receptors can, even in the absence of HIV-1 entry and replication, result in the activation of signal transduction pathways leading to chemokine expression. These soluble mediators, produced by both infected cells and bystander uninfected cells triggered by viral products (i.e., Tat and gp120), may regulate the course of HIV infection by either directly controlling the extent of viral infection or through their chemoattractive effect on immune cells. The production of chemokines may represent a protective response of macrophages to HIV infection and contribute to limit viral spreading by blocking specific coreceptor usage in uninfected cells. At the same time, these factors may play an important role in the HIV immune response by recruiting specific immune cell populations. In contrast, hyperproduction of chemokines during the course of infection may enhance viral spreading, favoring the infection of newly recruited immune cells, thus contributing to the pathogenesis of HIV infection. Although the in vivo biological relevance for the enhanced chemokine production remains to be elucidated, it is reasonable to assume that the balance of their negative vs positive effects on HIV spreading may contribute to different outcomes of HIV disease.

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